SEROLOGY OF SYPHILIS BASED ON RECENT OBSERVATIONS*†

BY

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Physicians and serologists have faced a number of questions associated with sero-diagnostic reactions in syphilis which have been difficult to answer. For example, it may be asked how is it possible for serum reactions employing lipid antigens, apparently unrelated to the *Treponema pallidum*, to behave as though they were specifically associated with syphilis, as shown by the remarkable parallelism between serologic and clinical findings in this disease ? Also, how can the same serum reactions behave as though they were totally unrelated to syphilis, as shown by the instances of biologic false positives noted in such a wide variety of non-syphilitic conditions ?

These two basic questions, as well as some others, are answerable according to recent observations made in this laboratory. As a first step it is necessary to recognize that the serology of syphilis is not a field complete in itself but a branch of a more comprehensive field of serology with lipid antigen. Some 30 years ago when we reported a test for syphilis based on precipitation (Kahn, 1923), we believed that the reaction between serum and lipid antigen was limited to syphilis ; that any reactions obtained in the absence of syphilis were due commonly to technical error or perhaps to some deficiency of sero-diagnostic tests ; that the very nearly perfect test would be free from false positives.

More recently it was reported from this laboratory (Kahn, 1947) that the same lipid antigens (Kolmer, cardiolipin, Kahn) which generally give specific

results in sero-diagnostic techniques for syphilis, may in special non-sero-diagnostic techniques give nearly 100 per cent. positive reactions in normal individuals. In due time, the universal serologic technique with Kahn antigen was standardized (Kahn, 1950a, 1951a). This technique has given positive reactions in all persons tested thus far, both in health and in disease.

To better understand sero-diagnostic reactions, whether in the presence or the absence of syphilis, it is essential to understand universal serologic reactions. Accordingly, before discussing the multiplicity of problems associated with sero-diagnostic reactions, consideration will be given to the universal serologic reaction in health, in syphilis, in some non-syphilitic diseases, and to the relationship between that reaction and the sero-diagnostic reaction.

Universal Serologic Reaction

The relation between the universal reaction which is multi-quantitative and the Kahn quantitative reaction is best understood by examining the basic techniques of the two reactions. In the Kahn quantitative technique, serial serum dilutions are prepared with 0·9 per cent. NaCl solution ; each dilution is mixed with standard Kahn antigen suspension, the mixtures are shaken for 3 minutes, and the flocculation results are read. To demonstrate the universal technique (simplified), seven quantitative set-ups are employed, consisting of serial serum dilutions prepared with different percentages of NaCl solution. The first serial serum dilutions are prepared with water, the second with 0·15 per cent., the third with 0·6 per cent., the fourth with 0·9 per cent., the fifth with 1·2 per cent., the sixth with 1·8 per cent., and the seventh with 2·1 per cent. These serial dilutions of serum are then appropriately

mixed with Kahn antigen suspension, and shaken for 3 minutes ; the first reading of flocculation results is made immediately, the second after 4 hrs' refrigeration at 5° C., and the third after 24 hrs' refrigeration. Employing this multi-quantitative technique, all normal human beings (and animals) tested have been found to give flocculation results of varying potency.

The universal reaction is performed with clear, un-contaminated blood serum which has been previously heated for 30 minutes at 56° C. After heating, the serum is permitted to stand for 10 minutes at room temperature before using. If frozen serum (from the deep freeze) is employed, it should be allowed to stand at room tem-perature until it is completely thawed, after which the tube should be stoppered and inverted gently several times to mix the contents thoroughly before placing it in the water-bath for the 30-minute heating period. (If for some special reason the heated serum is to be employed in subsequent examinations, it should be reheated for 10 minutes if retesting is to be carried out within 2 to 24 hrs of the initial heating period, or for 15 minutes if the retesting is carried out more than 24 hrs after the initial heating period.) Sera that are cloudy after the heating period may be cleared by the addition of a " pin-head " amount of talc or kaolin followed by centrifugation.

Preparation of Serial Dilutions of Serum

(1) Set up seven rows of tubes (in racks) with nine tubes in each row.

(2) Measure 0·15 ml. amounts of distilled water into Tubes 2 to 9, 1st row.

(3) Measure 0·15 ml. amounts of 0·15 per cent. NaCl solution into Tubes 2 to 9, 2nd row.

(4) Measure 0·15 ml. amounts of 0·6 per cent. NaCl solution into Tubes 2 to 9, 3rd row.

(5) Measure 0·15 ml. amounts of 0·9 per cent. NaCl solution into Tubes 2 to 9, 4th row.

(6) Measure 0·15 ml. amounts of 1·2 per cent. NaCl solution into Tubes 2 to 9, 5th row.

(7) Measure 0·15 ml. amounts of 1·8 per cent. NaCl solution into Tubes 2 to 9, 6th row.

(8) Measure 0·15 ml. amounts of 2·1 per cent. NaCl solution into Tubes 2 to 9, 7th row.

(9) Measure 0·15 ml. amounts of undiluted serum into Tube 1 of each of the seven rows.

(10) Add 0·15 ml. of the undiluted serum to Tube 2 of each of the seven rows.

(11) In each row, mix contents of Tube 2 by drawing up in pipette several times, and transfer 0·15 ml. to Tube 3, and in similar manner, transfer 0·15 ml. amounts serially, discarding the 0·15 ml. from the last tube. Each tube should contain 0·15 ml. amounts of the serially diluted serum.

(12) Tubes 6 and 8 of each row are not essential to the serologic pattern of the method, and are discarded.

(13) The set-up now consists of seven rows of tubes, each row containing undiluted serum and 1:2, 1:4, 1:8, 1:16, 1:64, and 1:256 dilutions of the serum.

Preparation of Kahn Antigen Suspension (as for the standard Kahn test)

(1) Measure into an antigen suspension vial the amount of saline, according to titre, required for the given amount of antigen.

(2) Measure into a second antigen suspension vial the necessary quantity of Kahn standard antigen.

(3) Pour the saline into the antigen and, without stopping, pour the mixture back and forth twelve times, without allowing vials to drain during the mixing period.

(4) Allow the antigen suspension to stand for 10 minutes before using.

(5) Discard antigen suspension after it has aged beyond 30 minutes. (A complete universal test requires about 1·5 ml. of antigen suspension. Since the minimum amount of antigen to be mixed with NaCl solution is 1·0 ml. it is well to mix antigen for two or three universal tests at one time, provided the worker can complete the tests before the 30-minute expiration period.)

Performance of Test

(1) Place thumb over mouth of suspension vial and shake briskly to suspend antigen suspension particles.

(2) Add 0·025 ml. amounts of this antigen suspension to each tube by touching the pipette to the inner wall of the tube and as closely as possible to the serum dilutions without actually making contact with them.

(3) Shake each rack by hand for 10 seconds imme-diately after the antigen suspension has been added to all tubes in that rack.

(4) After antigen suspension has been added to all the tubes, shake the racks for 3 minutes in a Kahn shaking machine (operating at a speed of 275 to 285 oscillations per minute with a 1½ inch stroke).

(5) Take the racks from the shaker and add :

    0·3 ml. distilled water in first row of tubes

    0·3 ml. 0·15 per cent. NaCl solution in second row of tubes

    0·5 ml. 0·6 per cent. NaCl solution in third row of tubes

    0·5 ml. 0·9 per cent. NaCl solution in fourth row of tubes

    0·5 ml. 1·2 per cent. NaCl solution in fifth row of tubes

    0·5 ml. 1·8 per cent. NaCl solution in sixth row of tubes

    0·5 ml. 2·1 per cent. NaCl solution in seventh row of tubes

(6) Shake rack by hand for about 5 seconds to mix the contents of the tubes.

(7) Read and record results. (The reading and record-ing scale is the same as in the Kahn test, namely : negative, doubtful (±), one plus, two plus, three plus, and four plus.)

(8) Place racks in ice-box immediately after reading results. (The ice-box temperature should be between 4° and 5° C.)

(9) After 4 hrs' incubation in the ice-box, remove racks one at a time, give each rack three sharp shakes by hand to dislodge any floccules adhering to tube walls, and read results as swiftly as is consistent with accuracy. (Exposure of the tubes to room temperature for too long a period may cause some of the flocculation reactions to disappear.)

(10) Again place racks in ice-box immediately after reading and reread results 20 hrs later according to the same procedure as after the 4-hour incubation period.

(11) The records of the results are then condensed into a graphic presentation. In plotting the results, flocculation readings of 2 plus, 3 plus, or 4 plus are considered positive, while 1 plus and doubtful results are considered negative. For an illustrative record of universal results plotted in graph form, see Figs 1-7.

General Considerations

It is assumed that workers will not attempt to do universal reactions unless they have had some experience in performing and reading serologic tests. Workers should perform a sufficient number of universal reactions to assure themselves of constancy in every step of the technique. The same serum specimen should give the same results on repeat examinations, whether in the hands of one worker or in the hands of different workers. If the results are not the same, then the difficulty lies perhaps in the measurements of the serum dilutions or of the antigen suspension, or in the reading of the results. Watchfulness of the technical steps of the universal reaction is of great importance because we are dealing with a colloidal reaction which is greatly affected by variations in such steps.

With regard to controls, since all sera react to some extent with antigen suspension on cold incubation, no serum-antigen suspension control is possible. In this laboratory we have employed two controls :

(*i*) a serum control

(*ii*) an antigen suspension-salt solution control

(*i*) The serum control consists of serial serum dilutions prepared in the same way as in the test, followed by shaking for 3 minutes, addition of amounts of diluents corresponding to those added to the test, incubation overnight at ice-box temperature, and examination for the presence of flocculation. The results have been invariably negative thus far.

(*ii*) The antigen suspension-salt solution control consists of mixtures of 0·025 ml. of antigen suspension with the various solutions of different salt concentrations in 0·15 ml. amounts, plus shaking for 3 minutes, the addition of diluents corresponding to those employed in the test, and incubation overnight at cold temperature. The results here also were almost invariably negative. Now and then it has been observed that in the higher salt concentrations (1·8 and 2·1 per cent.) some cloudiness or slight precipitation is observed, evidently due to the interaction between the lipids and the salt. But in those instances many sera did not give flocculation reactions which followed the pattern indicated by the antigen-salt solution mixtures, undoubtedly due to the protective action of the serum proteins.

Based on these findings we do not consider it necessary to have a set of controls for each universal test, but we consider it important to run controls (*i*) and (*ii*) from time to time especially when employing new lots of antigen, in order that workers may become fully familiar with the results of the control system.

NOTES ON GLASSWARE AND REAGENTS.—For uniformity in results, standard glassware and reagents should be employed as far as possible.

The glassware should be chemically clean (free from traces of acid or alkali). The test tubes should be clear and not markedly scratched, otherwise the presence of flocculation may be obscured in reading the results.

Vials.—Flat-bottomed for preparation of the antigen suspension, 5·5 cm. in length and 1·5 cm. in diameter.

Test Tubes.—7·5 cm. in length and 1·0 cm. in inner diameter.

Pipettes.—1·0 ml. (or 2·0 ml. optional) graduated in 0·01 ml. for measuring the NaCl solutions and the undiluted serum ; 0·2 ml. graduated in 0·01 ml. for preparing the serial dilutions of the serum ; 1·0 ml. for measuring the standard antigen, and 1·0 or 2·0 ml. for measuring the 0·9 per cent. NaCl solution in the preparation of the antigen suspension ; 0·25 ml. graduated in 0·0125 ml., or 0·2 ml. graduated in 0·001 ml. for measuring the antigen suspension.

Sodium Chloride.—Reagent quality NaCl should be employed. In preparing the NaCl solutions, chemical cleanliness rather than sterility is essential.

Antigen.—Kahn Standard antigen, properly standardized, is employed.

Results

Table I illustrates a universal serologic reaction

TABLE I

UNIVERSAL SEROLOGIC REACTION WITH NORMAL HUMAN SERUM

Seven Quantitative Set-ups of Serum Dilutions with Lipid Antigen

Readings	Serial Dilutions of Serum	Per cent. NaCl solutions employed in serum dilutions						
		0	0·15	0·6	0·9*	1·2	1·8	2·1
Without incubation of serum-antigen suspension mixtures	1 : 256	—	—	—	—	—	—	—
	1 : 64	—	—	—	—	—	—	—
	1 : 16	—	—	—	—	—	—	—
	1 : 8	—	—	—	—	—	—	—
	1 : 4	—	—	—	—	—	—	—
	1 : 2	±	—	—	—	—	—	—
	Undiluted	1	—	—	—	—	—	—
After 4 hrs' incubation in ice-box	1 : 256	1	—	—	—	—	—	4
	1 : 64	2	±	—	—	—	2	4
	1 : 16	4	4	—	—	—	3	4
	1 : 8	4	4	—	—	—	4	4
	1 : 4	4	4	—	—	—	4	4
	1 : 2	4	4	—	—	—	4	4
	Undiluted	4	4	—	—	—	4	4
After 24 hrs' incubation in ice-box	1 : 256	4	±	—	—	—	2	4
	1 : 64	4	3	—	—	—	3	4
	1 : 16	4	4	—	—	—	4	4
	1 : 8	4	4	—	—	±	4	4
	1 : 4	4	4	—	—	±	4	4
	1 : 2	4	4	—	—	2	4	4
	Undiluted	4	4	—	—	4	4	4

*The quantitative set-up with 0·9 per cent. NaCl solution, read without incubation, is similar to a quantitative Kahn reaction.

given by a normal individual. No flocculation is noted on the reading of the results previous to incubation of the serum-antigen suspension mixtures. After 4 hrs at a cold temperature some flocculation is noted in the quantitative set-ups of low NaCl concentration in which serial serum dilutions of 0 and 0·15 per cent. NaCl solutions are employed, and in the quantitative set-ups of high salt concentration in which serial serum dilutions of 1·8 and 2·1 per cent. NaCl solutions are employed. After 24 hrs at a cold temperature, a small increase in flocculation is noted. The plotted flocculation results in the Table are presented in Fig. 1. The Figure consists of three sections in which the cross-hatched areas represent the flocculation results. Only $++++$, $+++$, and $++$ readings are plotted in the Figure, $+$ and $\pm$ readings being classed with negative reactions. The general contour of these cross-hatched areas is referred to as the *serologic pattern*. All the Figures presented in this article are constructed in the same way as Fig. 1.

It is evident that one of the seven quantitative set-ups which make up the universal serologic technique is equivalent to a quantitative Kahn test ; namely, the one that employs serial dilutions of serum with 0·9 per cent. NaCl solution, and in which the flocculation results are read without incubation. This quantitative set-up generally shows no flocculation in normal individuals. It shows flocculation in syphilis and in certain other situations

and is represented in the figures by a double cross-hatched column, so that it may be easily identified.

Universal Serologic Reaction in Health exemplifying a Non-Specific Reaction.—Graphs 1, 2, and 3 of Fig. 2 (overleaf) illustrate universal reactions in three individuals in health. The degree of flocculation in Graph 1 is considered weak. Approximately the same degree of flocculation represents the most common form of universal reactions noted in health in the midwestern part of the United States. The degree of flocculation in Graph 2 is considered moderate, and in Graph 3 strong. The serologic pattern illustrated in these graphs is considered to be the result of non-specific lipid antigen-antibody reactions.

A characteristic of the universal reaction in health is the tendency towards increased flocculation following incubation at a cold temperature. Thus, usually no flocculation is noted in the first section of the graph in which the results are read without incubation. Some flocculation makes its appearance after 4 hrs' incubation and is increased after 24 hrs' incubation.

Universal Serologic Reaction in Syphilis, exemplifying a Lipid-Specific Reaction.—Graphs 4, 5, and 6 of Fig. 2 illustrate three universal reactions in syphilis. The double cross-hatched columns in the graphs represent quantitative sero-diagnostic reactions and will be considered below. An examination
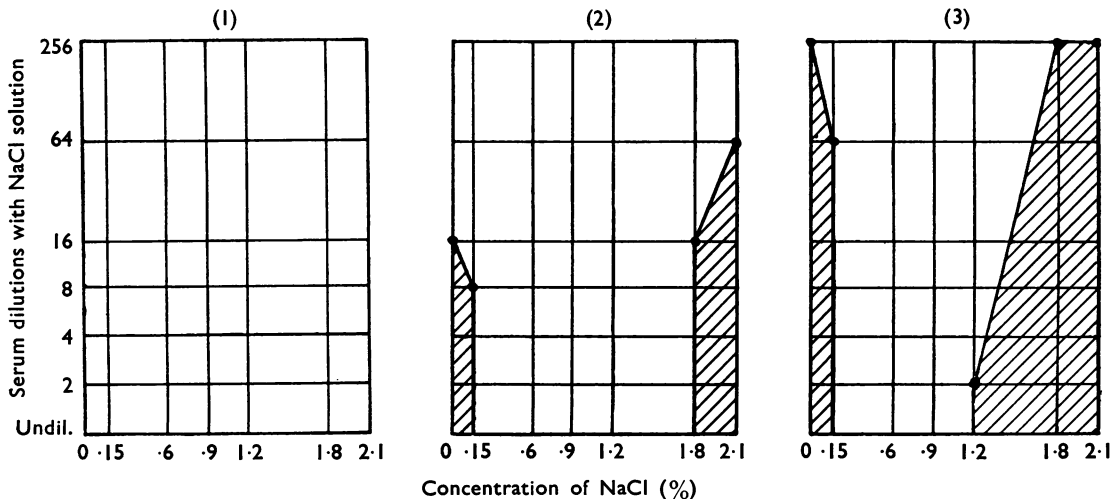

FIG. 1.—Universal reaction presented in three sections based on three readings of precipitation results recorded in Table I :

(1) without incubation,
(2) after 4 hrs' incubation,
(3) after 24 hrs' incubation.

All the other graphs in this article are constructed in the same manner.

IN HEALTH   IN SYPHILIS

Graph 1   Graph 4

Graph 2   Graph 5

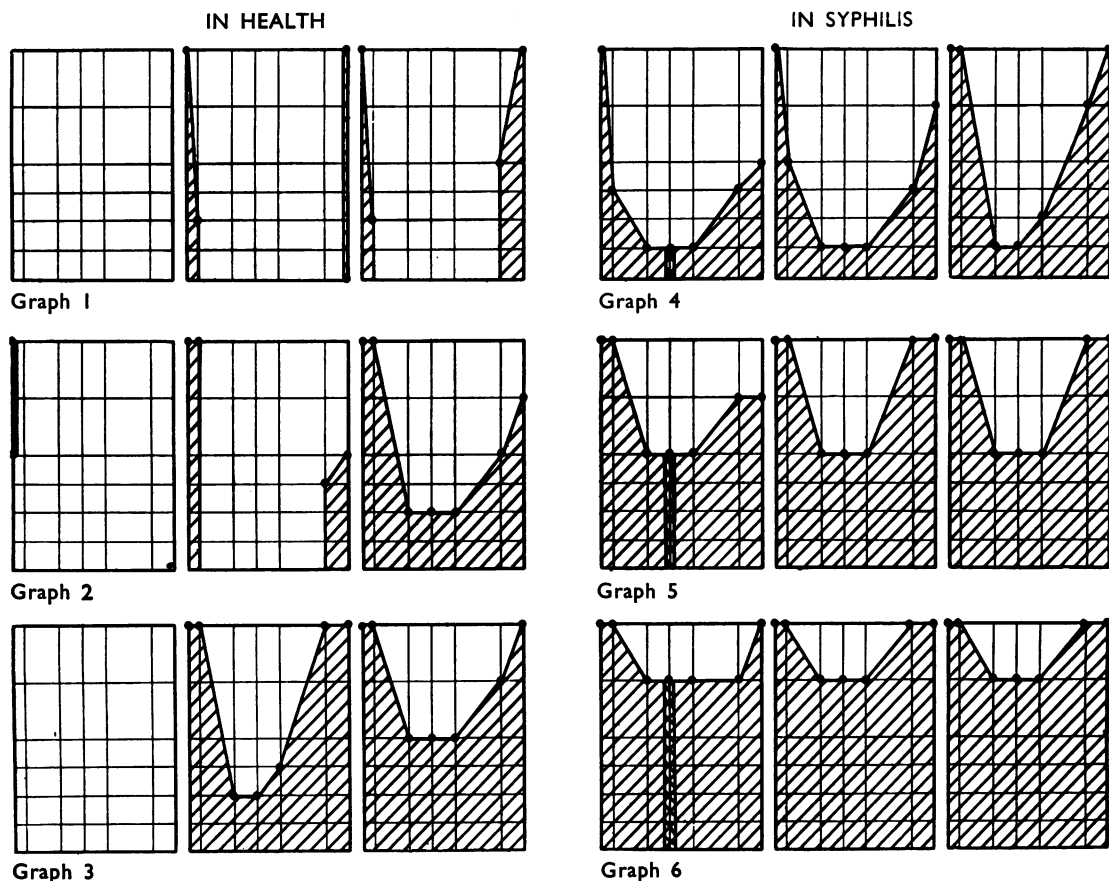Graph 3   Graph 6

FIG. 2.—Graphs 1, 2, and 3 represent weak, moderate, and strong universal reactions of normal human beings ; the reactions are considered non-specific. Graphs 4, 5, and 6 represent weak, moderate, and strong universal reactions in syphilis ; the reactions, being distinctive, are considered to be lipid-specific. The double cross-hatched columns represent quantitative sero-diagnostic reactions.

of the serologic pattern illustrated in these graphs reveals two distinctive characteristics in which it differs from the other serologic patterns seen in this article. In other graphs, as already indicated, there is a tendency toward increased flocculation following cold incubation, but in Graphs 4, 5, and 6 there is no such tendency. Another feature of the graphs is the uneven central flocculation zone before and after cold incubation, while in syphilis the central zone remains at the same level.

The distinctive serologic pattern of the universal reaction in syphilis must be the result of a distinctive lipid antigen-antibody reaction, based on the union between particular lipids and homologous antibodies. Apparently, therefore, the universal reaction in syphilis is a lipid-specific antigen-antibody reaction. Hence, the sero-diagnostic reaction in syphilis, represented by the double cross-hatched columns in Graphs 4, 5, and 6, given by one of the seven quantitative procedures, must necessarily also be a lipid-specific antigen-antibody reaction.

It should be added that the serologic pattern of the universal reaction in yaws, pinta, and bejel is similar to the pattern in syphilis (Kahn and Gutierrez Villegas, 1952). Hence, the universal and sero-diagnostic reactions in these treponemal diseases are also considered to be lipid-specific. At one time we believed the serologic pattern in yaws to be somewhat different from that in syphilis (Kahn 1950b). The basis for that belief was that we compared reactions in yaws, given by sera received from Jamaica, with reactions in syphilis given by sera obtained in Michigan. In the study of any differences in characteristics of universal reactions in syphilis, yaws, pinta, and bejel, the comparative studies must be carried out in the same geographic area.

The relationship between the non-specific universal reaction in health and the lipid-specific reaction in syphilis is illustrated in Fig. 3. This figure was taken from a report by Curtis, Rowe, Baribeau, and Kahn (1951) on the universal reaction in experimental rabbit syphilis. Indications are that the normal universal reaction is not affected in syphilis ; that a new lipid-specific reaction makes its appearance in this disease which masks the normal reaction. With appropriate therapy, the syphilis reaction disappears, bringing the normal universal reaction to light again.
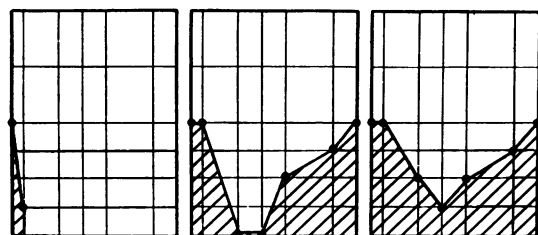
Graph I   June 29, 1948.   Control
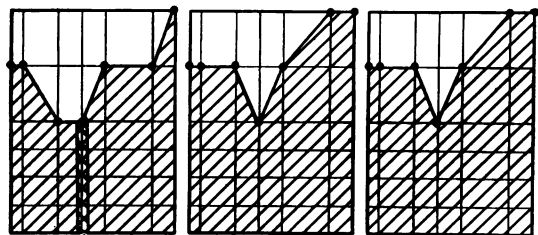
Graph 2   Sept. 20, 1948.   82 days after inoculation
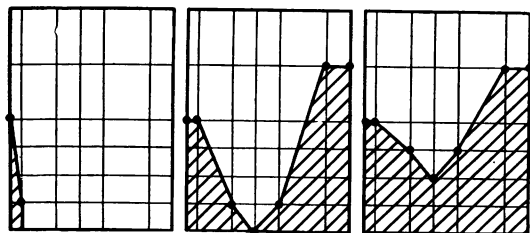
Graph 3   Nov. 19, 1948.   42 days after treatment

FIG. 3.—Universal and sero-diagnostic reactions in rabbit syphilis. Lipid antigen-antibody reactivity is increased in syphilis, but this increase is not the result of the intensification of the normal reaction but of a distinctive reaction associated with syphilis. (Curtis and others, 1951.)

Universal Serologic Reaction in the Lepromatous Form of Leprosy, also exemplifying a Lipid-Specific Reaction.—Fig. 4 (overleaf) illustrates universal reactions in the lepromatous form of leprosy. Flocculation in the first sections of the graphs in which the results are read without incubation is strongest in the zone of low NaCl concentra-

tion. After 4 and 24 hrs' incubation, flocculation is increased again, mainly in the zone of low salt concentration and in the central zone. Graphs 1, 2, and 3 represent universal reactions of three selected patients with leprosy who have shown improvement with time. Graphs 1A, 2A, and 3A represent the reactions at later dates after their improvement had been established clinically (Kahn, 1951b ; Kahn, Thiers-Pinto, and Zeo, 1951).

These three patients were chosen to demonstrate that lipid antigen-antibody reactivity in leprosy (as in syphilis) is reduced with improvement. It is believed that the serologic pattern illustrated by graphs 1A, 2A, and 3A in Fig. 4 is typical of the lepromatous form of leprosy. The pattern is not seen in all instances, because in this form of the disease concurrent diseases are often encountered which mask the typical pattern. Yet the fact that such a pattern is noted in many instances would indicate that a distinctive lipid antigen-antibody reaction is manifested in this form of leprosy. Hence, the universal as well as the sero-diagnostic reactions are considered to be lipid-specific.

Universal Serologic Reaction in Malaria, exemplifying a Non-Specific Reaction.—In vivax malaria, the serologic response of the universal reaction is neither like the one noted in syphilis nor like the one in leprosy. The serologic pattern in malaria, illustrated in Fig. 5 (overleaf), is not considered distinctive and appears to be the result of a quantitative intensification of the normal serologic response. Graph 1 illustrates a universal reaction in a normal individual ; Graph 1A an intensified reaction some 2 weeks after a febrile attack of vivax malaria, and Graph 1B a reversal to the normal universal reaction. Graphs 2, 2A, and 2B represent similar reactions in another individual. The double cross-hatched column in Graph 2A represents a quantitative sero-diagnostic reaction. Fig. 5 shows first that in malaria the increase in the intensity of the lipid antigen-antibody reactivity is apparently not specific, and secondly that this increase in one individual may be such that flocculation in the universal reaction is sufficiently marked to reach the sero-diagnostic zone and lead to a positive sero-diagnostic reaction, while in another individual the increase may not be sufficiently marked for flocculation to reach the sero-diagnostic zone.

Universal Serologic Reaction in Tuberculosis, exemplifying a Non-Specific Reaction.—Fig. 6 (overleaf) illustrates universal reactions in a normal individual (Graph 1), an intensified reaction as a result of oncoming tuberculosis (Graph 1A), and a reversal

INITIAL EXAM

IMPROVEMENT

Graph I　Nov. 17, 1947

Graph IA　Oct. 3, 1951

Graph 2　Sept. 22, 1947

Graph 2A　Oct. 1, 1951
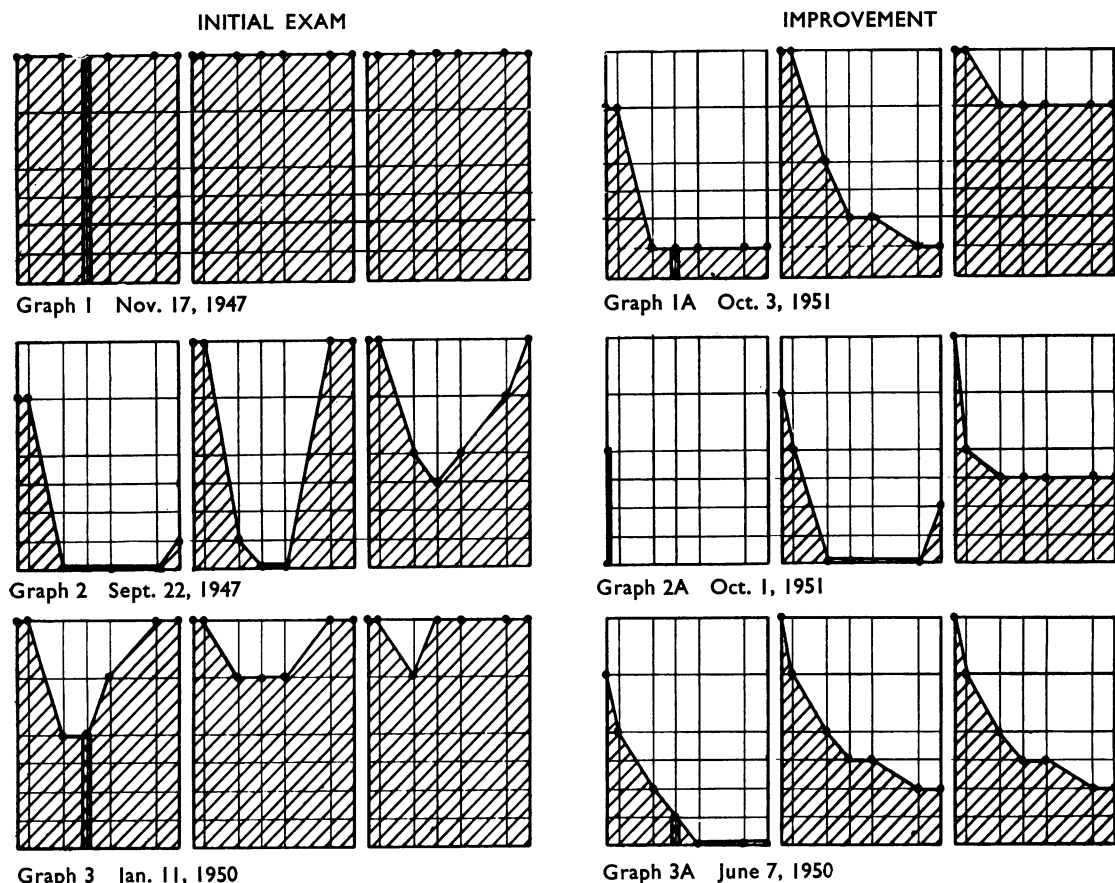
Graph 3　Jan. 11, 1950

Graph 3A　June 7, 1950

FIG. 4.—Universal and sero-diagnostic reactions in leprosy. Graphs 1, 2, and 3 represent strong universal reactions with positive sero-diagnostic reactions (double cross-hatched columns). Graphs 1A, 2A, and 3A represent universal reactions of reduced reactivity and weaker sero-diagnostic reactions associated with improvement.

Graphs 1–1A, and 2–2A were kindly supplied by Sister Hillary of the U.S. Public Health Service Hospital at Carville, La.

Graphs 3 and 3A were taken from the report by Kahn, Thiers-Pinto, and Zeo (1951).

The improved patients show distinctive patterns of lepromatous leprosy.

to very nearly the normal response 10 months later (Graph 1B). As in malaria, the reaction in tuberculosis is believed to represent a non-specific intensification of a normal reaction. An outstanding feature of this intensification was that it was observed about 3 months before the establishment of a diagnosis of tuberculosis in this individual. It is of interest that while in vivax malaria there is a tendency for flocculation in the universal reaction to spill over into the sero-diagnostic zone, in tuberculosis this tendency is manifested but rarely ; Graph 1A in Fig. 6, in which flocculation almost reached the sero-diagnostic zone, may be looked upon as an exception to the general rule. Hence, in tuberculosis, it is quite uncommon to obtain positive reactions with sero-diagnostic tests for syphilis. In

an evaluation of such tests in 444 patients with tuberculosis carried out by the United States Public Health Service and reported by Parran and Emerson (1939), not a single false positive Kahn reaction was reported from this laboratory.

How can Qualitatively Different Serologic Patterns be obtained with a Single Serologic Technique ?— Three qualitatively different serologic patterns of universal reactions are presented in Figs 2 to 4—the patterns in normal individuals, in syphilis, and in the lepromatous form of leprosy. These three qualitatively different patterns are believed to be the result of three antigenically different lipids present in the antigen, reacting with their homologous antibodies present in the sera, in health, in

Graph I   Feb. 10, 1948

Graph 2   Feb. 10, 1948

Graph IA   March 10, 1948

Graph 2A   March 10, 1948

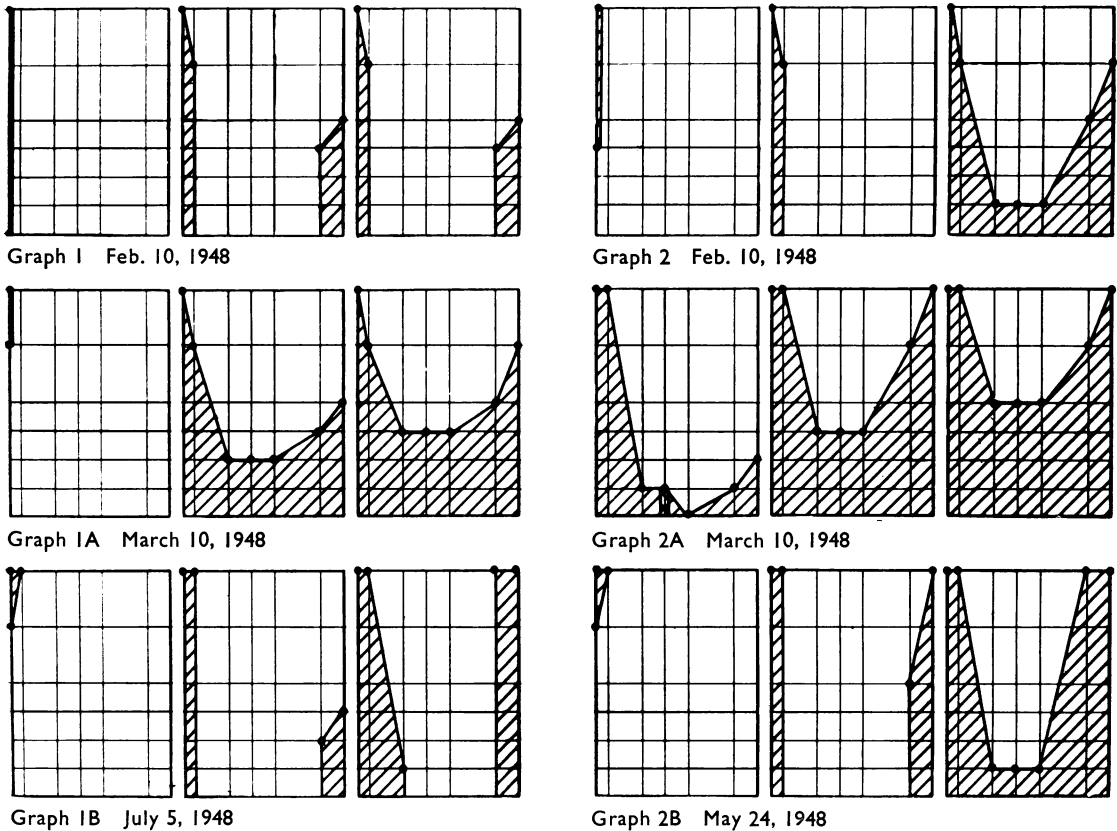Graph IB   July 5, 1948

Graph 2B   May 24, 1948

FIG. 5.—Universal and sero-diagnostic reactions in malaria.   Graph 1 represents a universal reaction obtained on the date of inoculation (when individual was bitten by infected mosquitos).   Graph 1A represents reaction obtained about 2 weeks following the primary febrile attack.   Graph 1B represents universal reaction following therapy and recovery 4 months later ; reaction is then similar to the original reaction on date of inoculation.
Graphs 2. 2A, and 2B represent universal reactions given by another individual under the same conditions.
The short double cross-hatched column in Graph 2A represents a quantitative sero-diagnostic reaction.

syphilis, and in leprosy. Thus, what might be called Lipids *N* react with antibodies *n* present in normal serum, Lipids *S* react with antibodies *s* present in syphilitic serum, and Lipids *L* react with antibodies *l* present in the serum in leprosy.

These and other antigenic lipids are believed to be present in all lipid antigens employed in syphilis tests. If these antigens contained only the antigenic lipids capable of reacting with syphilitic antibodies, there would be no false positive problem in the serology of syphilis. Actually, the antigens used in the Kolmer test, in a cardiolipin test, in the standard Kahn test, or in other tests, contain multiple antigenic lipids, each capable of reacting with homologous antibodies.   These antibodies, with the exception of the syphilitic variety, are not favourable for detection by sero-diagnostic tests, but they are favourable for detection by the universal serologic technique. As a result, sero-diagnostic reactions are negative in instances in which universal reactions are positive.

Not all diseases show an increase in reactivity of the universal reaction over the normal reactivity level.   It is believed that one disease may show markedly increased reactivity of the universal reaction, another disease but slightly increased reactivity, and still another no increase because of the lipid constitution of the antigen used in the universal reaction. Thus, in one disease the appropriate lipids are present in optimal quantity in the antigen, in another they may be present in partial degree, and in yet another they may be totally absent. When, for example, the universal reaction with Kahn antigen was applied in this laboratory to the study of sera from cancer patients, no increased reactivity was observed over the normal level ; but,
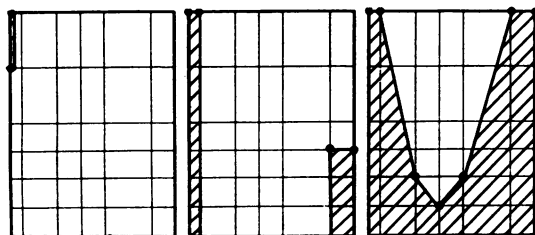
Graph I Apr. 11, 1947
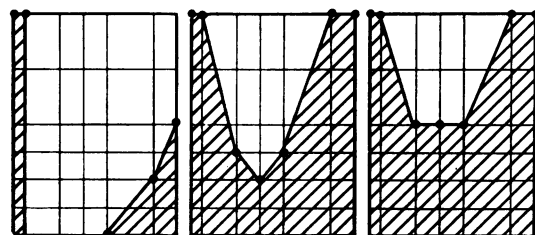
Graph IA Dec. 11, 1947
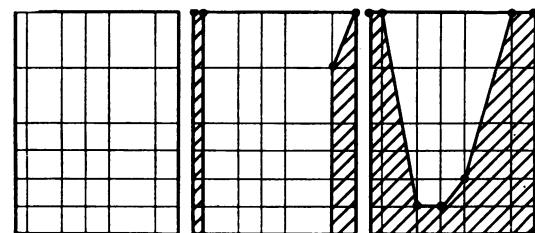
Graph IB Nov. 3, 1948

Fig. 6.—Universal serologic reactions in tuberculosis. Graph 1 represents a universal reaction obtained in a normal individual. Graph 1A represents a universal reaction obtained 8 months later in the same individual, who though believed to be normal was diagnosed as tubercular 2 months later still. The universal reaction after the individual had been hospitalized for 10 months and discharged is represented by Graph 1B.

with a modified antigen, some increased reactivity was noted. This increase is non-specific and has no diagnostic value, but it indicates that the presence of appropriate lipids in the antigen results in increased reactivity of the universal reaction in a given disease.

Sero-Diagnostic Reactions in Syphilis, Leprosy, Malaria, and Tuberculosis

Clinical Value of Sero-Diagnostic Reactions in Syphilis.—If the reaction between serum and lipid antigen is biologically universal, why is it that, out of several chronic diseases here considered, sero-diagnostic reactions are of clinical value in only one ? Four factors may help to explain the clinical value of sero-diagnostic reactions in syphilis and their lack of clinical value in leprosy, malaria, and tuberculosis.

Factor 1.—In syphilis, as was already seen, the sero-diagnostic reaction is lipid-specific ; the specificity being directed not against the *T. pallidum*, but against particular lipids set free presumably in pathologic processes in syphilis. The distinctive serologic pattern of the universal reaction in syphilis is interpreted to mean that distinctive lipids associated with this disease—liberated most likely as a result of tissue break-down—presumably undergo chemical changes or conjugations which make them foreign to the body and auto-antigenic, and these lipids call forth homologously distinctive auto-antibodies.

The lipid-specific nature of the reaction between serum and lipid antigen in syphilis is not manifested by the technically restricted sero-diagnostic tests ; the reactions given by these tests both in the presence and in the absence of syphilis appear to be the same. It is the more comprehensive universal serologic technique which brings out the distinctive serologic pattern in syphilis.

Factor 2.—Syphilis, being a disease of moderate activity, calls forth high-level antibody production. Moderate activity of any disease often goes hand-in-hand with marked antibody production, while minimal activity and excessive activity are generally forerunners of low antibody production. Excessive tissue break-down in syphilis, under the name of malignant syphilis, used to be encountered now and then in years gone by, and the serologic reactions were either negative or very weakly positive.

Factor 3.—In syphilis, antibody production to lipids is not short-lived, as in acute infections, but is produced continually because of the chronic nature of the disease. In latent syphilis, the serologic response would indicate that subclinical syphilitic processes in persons in whom the disease has existed for many years are sufficient for antibody production. The prolonged existence of an antibody-producing disease apparently develops the capacity for antibody production following minimal antigenic stimulus. Serologic fastness (persistent positivity) following adequate therapy is particularly difficult to explain. The immunologic mechanism involved may be the same as in the serologic fastness of specific reactions, such as the agglutination reaction of typhoid bacilli following recovery from typhoid fever.

Factor 4.—In syphilis, the techniques of sero-diagnostic tests are believed to be very nearly optimal for eliciting positive reactions. If the antigen employed in the standard Kahn test is taken

as an illustration, it will be found that the increase in concentration of the lipids in the antigen, or the reduction of lipids by dilution of the antigen with alcohol, will reduce the sensitivity of the Kahn test with syphilitic serum, which suggests that the antigen is used under optimal conditions in syphilis. It is, of course, possible to increase the sensitivity of antigens beyond the level of sero-diagnostic tests, but that step would lead to an increase in the number of positive reactions in the absence of syphilis.

Lack of Clinical Value of Sero-Diagnostic Reactions in Leprosy.—It was seen that universal and sero-diagnostic reactions in the lepromatous form of leprosy are lipid-specific. It might therefore have been expected that sero-diagnostic reactions would give valuable results clinically in this form of the disease. Actually, sero-diagnostic reactions are of some clinical value in certain types of patient—those in whom the disease is in a moderately active state. In these patients antibody production is at a high level, and they give high quantitative sero-diagnostic titres which are reduced with improvement, as is evident from Fig. 4.

In those patients with the lepromatous form of leprosy in whom the activity is minimal or excessive, antibody production to lipids is at a low level, and sero-diagnostic reactions are generally negative. Sero-diagnostic reactions are also commonly negative in tuberculoid and non-characteristic forms of leprosy, presumably because of the minimal activity of the disease.

It appears, therefore, that in the lepromatous form of leprosy, lipid-specificity and chronicity of the disease tend to give clinical value to sero-diagnostic reactions, but the wide range in the activity of the disease interferes with the clinical value of these reactions. It is possible also that sero-diagnostic techniques are not as optimal for leprosy as they are for syphilis.

Lack of Clinical Value of Sero-Diagnostic Reactions in Vivax Malaria.—Sero-diagnostic reactions in this form of malaria are apparently not lipid-specific, universal serologic reactions showing merely intensification of normal reactions. Beginning about 2 weeks after the febrile attack, antibody production to lipids may reach a high enough level for sero-diagnostic reactions to become positive, but the increase in antibody production is generally of short duration. Relapses tend to reactivate antibody production, but, as the disease reverts to the quiescent state, the antibodies gradually disappear from the circulation. There are thus two main factors which interfere with any clinical value of sero-diagnostic tests in vivax malaria : the increase in serologic reactivity following febrile attacks may not reach the sero-diagnostic zone, and when it does the sero-positivity is of relatively short duration. It is possible also that, as in the case of leprosy, the techniques of sero-diagnostic tests are not optimal for vivax malaria.

Lack of Clinical Value of Sero-Diagnostic Reactions in Tuberculosis.—In early tuberculosis there is generally an increase in the reactivity of the universal serologic reaction over the normal reactivity level. But in most instances this reactivity does not become so marked as to reach the sero-diagnostic zone and give positive sero-diagnostic reactions. In far-advanced and miliary tuberculosis no intensification of the universal reaction has been noted, presumably because of the excessive activity of the disease. The universal reaction in minimal tuberculosis is yet to be investigated. Some patients with moderately advanced tuberculosis behave serologically like patients with early tuberculosis and show intensification of the universal reaction. Others behave serologically like patients with far-advanced tuberculosis and show no increase in intensity of the reaction.

Why does the reactivity of the universal reaction in early tuberculosis commonly fail to reach the sero-diagnostic zone ? The answer most likely is that the antigen employed in the universal reaction does not contain those particular lipids in such quantity as to react optimally in tuberculosis. Universal reactions of only partial strength are obtained, just as the employment of a " weak " antigen in a syphilis test commonly leads to weak or negative reactions. Individuals with early tuberculosis, who normally give universal reactions of such pronounced strength that the reactivity is close to the sero-diagnostic zone, are of course very likely to become positive reactors with sero-diagnostic tests.

False Positive Reactions in Miscellaneous Conditions

Definition.—False positive reactions are often looked upon as " incorrect " reactions given by tests for syphilis. Actually, they are no less biologically correct than syphilis reactions. They may be looked upon as universal serologic reactions obtained in various non-syphilitic conditions in which the reactivity is of such marked intensity as to " spill over " into the sero-diagnostic zone of syphilis tests. Universal reactions in which serologic

reactivity is removed from, is close to, or actually reaches, the sero-diagnostic zone (illustrated in Fig. 7) will be considered more fully later in connection with the question : Who might give false positive reactions ?

In Chronic Infections.—False positive reactions were considered in leprosy, malaria, and tuberculosis to illustrate the basis for the wide variations in lipid antigen-antibody reactivity existing in those three chronic diseases. Intensification of this reactivity over the normal level is noted in certain stages of these diseases, but only in special instances are positive reactions encountered with sero-diagnostic techniques. It is believed that all chronic infections may be found to show intensifications over the normal lipid antigen-antibody reactivity provided the right antigen or the right technique, or both, are employed. With regard to false positives, these may be encountered with syphilis tests in any disease in such persons who are normally strong lipid antigen-antibody reactors. Thus, in persons in whom the reactivity of the universal reaction is normally so strong as to be close to the sero-diagnostic zone, even a small increase in reactivity associated with disease may lead to positive sero-diagnostic reactions.

In Acute Infections.—False positive reactions are rarely encountered during acute infections, especially if they are of short duration. The reason is that it takes an incubation period of about 2 weeks for an increase to occur in the lipid antigen-antibody reactivity over the normal reactivity level. It is undoubtedly because of this incubation period that false positive reactions associated with acute infections are generally encountered after recovery. When the universal reaction was first observed, an attempt was made to find to what extent its reactivity is increased during febrile periods in acute infections. But the reactions obtained seemed very much like those given by normal individuals.

In Non-Infectious Diseases.—An outstanding feature of the increase in lipid antigen-antibody reactivity in disease over the normal level is that this increase is not limited to infectious diseases, but is manifested also in pathologic disturbances not associated with infection. Thus, it was already indicated that increased lipid antigen-antibody reactivity may be noted in cancer by the use of a specially prepared lipid antigen. Apparently pathologic processes resulting from any cause may increase lipid antigen-antibody reactivity, but this reactivity may cause sero-diagnostic reactions to become positive only in rare instances.

F.P. IMPROBABLE

(1)

F.P. PROBABLE

(2)
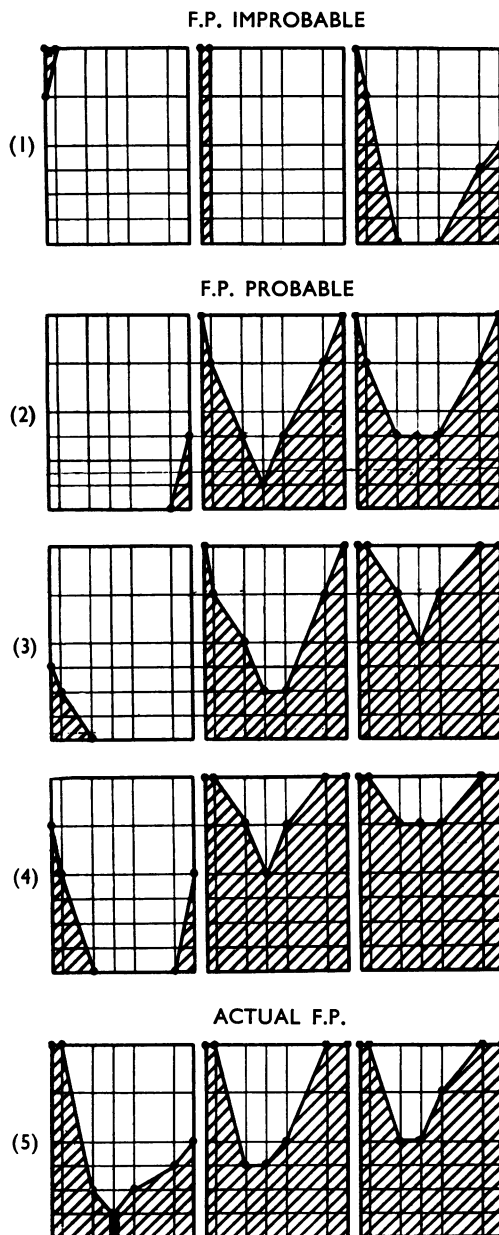
(3)

(4)

ACTUAL F.P.

(5)

Fig. 7.—Universal reactions as indications of tendency towards false positives. Individuals who normally give weak universal reactions (Graph 1) are not likely to give false positives because the reactivity would have to be markedly increased for it to reach the sero-diagnostic zone.
Individuals who normally give universal reactions (Graphs 2, 3, and 4) are likely to give false positives because their reactivity being close to the sero-diagnostic zone, even a small increase might cause it to reach that zone.
In Graph 5 the reactivity has actually reached the sero-diagnostic zone.

After Injections of Antigenic Agents.—Studies by Kahn, Wheeler, and Brandon (1951) and by Kahn and Petrenco (1951a) showed that several injections of horse serum or of killed tubercle bacilli in rabbits intensified the reactivity of the universal serologic reaction over the normal reactivity level of these animals. In some instances the intensification was so marked as to reach the sero-diagnostic zone and lead to a positive sero-diagnostic reaction; in others, the intensification was relatively slight.

In the case of the horse-serum injected rabbits, when specific precipitins were determined side by side with the acquired antibodies to lipids, some parallelism was observed in the appearance of these two types of antibodies. Since immunizing injections with antigenic agents in animals cause an increase in lipid antigen-antibody reactivity over the normal reactivity level, isolated instances are bound to arise in which immunizing injections in human beings may cause an increase in reactivity great enough to result in positive sero-diagnostic reactions.

After Injections of Non-Antigenic Agents.—Subcutaneous injections of tissue lipids (Kahn and Petrenco, 1951b) or of paraffin oil (Kahn, 1951c) in rabbits were found in this laboratory to cause intensification of the universal serologic reaction over the normal level. As in the animals injected with antigenic agents, the intensification in some instances was so marked as to lead to positive sero-diagnostic reactions. There is, therefore, no reason why similar instances of positive reactions may not occur in human beings after the injection of non-antigenic foreign substances.

After Irradiation.—False positive Kahn reactions after irradiation have not been observed in the Serology Laboratory of the University of Michigan Hospital, but increased reactivity of the universal reaction after irradiation is not uncommon and has been reported by Kahn, Hodges, Lampe, and Doyle (1952) and by Kahn, Bullock, and Bethell (1952). Evidently the reactivity is generally not sufficiently marked to reach the sero-diagnostic zone. But instances are bound to occur in naturally strong lipid antigen-antibody reactors in whom the reactivity may reach the sero-diagnostic zone, and lead to false positive reactions.

Before Clinical Manifestations.—In diseases of prolonged onset, positive sero-diagnostic reactions may precede the clinical manifestations. In a patient with tuberculosis the intensification of the universal reaction over the normal level, previous to clinical manifestations, has already been referred to

(Fig. 6), and it is possible that the reactivity in a given instance may reach the sero-diagnostic zone and lead to a positive sero-diagnostic reaction. Haserick and Long (1952) reported several cases of lupus erythematosus in which positive sero-diagnostic reactions were obtained before the disease was clinically apparent. Keeping a persistent false positive reactor under observation is undoubtedly the only way to determine whether the reaction is a forerunner of some chronic disease. Universal and sero-diagnostic studies of persons exposed to lepromatous leprosy may reveal a distinctive serologic pattern of this form of the disease and, in some instances, a positive sero-diagnostic reaction, before the clinical manifestations of the disease.

In Animals.—It is well known that different animal species give positive reactions with tests for syphilis (Kahn, 1940a). Thus, if the sera of cows, horses, pigs, sheep, rabbits, or chickens are examined with a sero-diagnostic test for syphilis, whether by precipitation or complement-fixation, positive reactions will be obtained in many instances. These reactions are apparently related to the biologic universality of serum reactions with lipid antigen. Serologically, the main difference between the animals listed above and normal human beings is that the animals tend to give stronger reactions with lipid antigen, and, when the universal technique is employed, the reactivity in many instances is strong enough to reach the sero-diagnostic zone.

In Normal Individuals.—In rare instances normal individuals may give false positive reactions. In these individuals the reactivity with lipid antigen is sufficiently marked in health to reach the sero-diagnostic zone. At the present time there is no way of differentiating serologically a false positive given by a normal individual from one given under conditions of disease. Perhaps the time will come when the universal reaction with an appropriate antigen will differentiate various types of false positives, based on differences in serologic patterns.

Fluctuating False Positives.—Physicians generally interpret a positive sero-diagnostic reaction in a non-syphilitic person, followed in a few days or longer by a negative reaction, as a technical false positive (as differentiated from a biologic false positive). Such a change in the sero-diagnostic reaction may be caused by the fact that the donor's lipid antigen-antibody reactivity is close to the sero-diagnostic zone and that his reactions are therefore likely to fluctuate from negative to positive and *vice versa*.

Residual False Positives.—The majority of false positive reactions encountered in practice are residual to some previous injection or infection. Once antibody production to lipids in a given individual is increased to the extent of giving a positive sero-diagnostic reaction, the antibodies will be circulating in the blood stream anywhere from some weeks to several months. A weak false positive reaction may mean that the reaction will become negative in the course of a few weeks. A strong false positive reaction may not reach negativity for several months.

Incidence of False Positive Reactions

Who are liable to give False Positives ?—The universal serologic reaction may indicate who is likely to give false positive reactions and who is not. Briefly, those who normally give universal reactions of low intensity are not so likely to give false positive reactions as those who normally give universal reactions of such high intensity as to be close to the reactivity zone of sero-diagnostic tests. Fig. 7 illustrates this relationship between universal reactions and false positive sero-diagnostic reactions.

Thus, it is believed that individuals giving such universal reactions as that illustrated in Graph 1 of Fig. 7, are not likely to give false positive reactions. These individuals will undoubtedly show increased lipid antigen-antibody reactivity under certain conditions, leading to more marked universal reactions. But for sero-diagnostic reactions to become positive, the increase in lipid antigen-antibody reactivity has to be unusually marked. Of interest is the fact that individuals giving stronger universal reactions, by showing strong flocculation limited to cold incubation, may not necessarily show a tendency toward false positive reactions. Such universal reactions are often seen in early tuberculosis ; yet false positive reactions in this disease are quite rare.

Individuals giving the universal reactions illustrated in Graphs 2, 3, and 4 of Fig. 7 are considered potential false positive reactors, since their lipid antigen-antibody reactivity is close to the sero-diagnostic zone. Therefore, any condition tending to increase this reactivity may easily cause it to actually reach the sero-diagnostic zone and result in positive sero-diagnostic reactions. Such individuals may give a positive sero-diagnostic reaction on one day and perhaps a negative reaction a day or two later ; or a positive reaction with one sero-diagnostic test and a negative reaction with another. The reason is that they show reactivity in the universal reaction which almost reaches the sero-

diagnostic zone. In Graph 5 of Fig. 7 the reactivity has actually reached the sero-diagnostic zone.

Incidence in Different Peoples.—Universal serologic reactions given by different peoples in different geographic areas of the world vary markedly in intensity (Kahn, McDermott, Finch, Hiler, and Reiss, 1953 ; Kahn, DeLien, and Wilder, 1953 ; Kahn, Fawkes, dos Santos, Gentle, and Yuen, 1953 ; Kahn and Singer, 1953). Thus, American Indians, East Indians, and Negroes living in Trinidad, " Cape Coloured " South Africans, and individuals in Uganda, show a far greater tendency to give moderate and strong universal reactions than midwestern Americans. The higher capacity for antibody production to lipids may manifest itself only on cold incubation and may not affect sero-diagnostic reactivity. But when this higher capacity is manifested also in the absence of incubation, the incidence of false positive sero-diagnostic reactions is likely to be higher than in midwestern Americans.

In undertaking serologic studies of different peoples by means of sero-diagnostic tests, it is important to first determine the base line of lipid antigen-antibody reactivity of these peoples. The universal reaction should prove an indicator of such reactivity. This reaction may also help in differentiating the lipid-specific serologic pattern in syphilis from the non-specific pattern in the absence of syphilis in a number of instances, but not in all ; the reason is that the strong universal reactions given by certain persons in health may mask the distinctive serologic pattern of syphilis if the same persons acquire this disease.

Biologic Basis of Sero-Diagnostic Reactions in the Presence and in the Absence of Syphilis

The biologically widespread nature of the reactions between serum and various lipid antigens has been recognized by many workers in the course of their search for a substitute for tissue extract antigens for syphilis tests. As an illustration, the early studies of Browning, Cruickshank, and M'Kenzie (1910) may be mentioned. They were interested particularly in the use of an alcoholic lecithin-cholesterol solution as a substitute for crude organ extract in the Wassermann reaction. Of historical importance is the fact that these authors were the first to employ cholesterol with tissue extract antigen in this test. They saw much reactivity between chemically prepared lipid antigens and sera from normal persons. But any such reactions were looked upon as " false positives ". In this connexion an extensive study by Mackie and Anderson (1937) may

be mentioned. These workers reported three different reacting zones with human and animal sera by the use of lipid suspensions from various sources (sheep heart, *B. diphtheria*, and lecithins). The two major factors in the differentiation of the zones were the heating of the sera at various temperatures and their dilution with salt solution. At a certain dilution of the sera, heated, let us say, to 60° C., a given precipitation zone was obtained which could be reproduced when the same conditions were adhered to. At another serum dilution, another precipitation zone was noted. The authors demonstrated the distinctive nature of the three reacting zones, by special absorption techniques. These experiments show the inherent capacities of sera, whether human or animal, for reacting with various lipid suspensions under different conditions.

One of the earliest theories which attempted to explain the basis of positive Wassermann reactions in syphilis was suggested by Weil and Braun (1909). They thought that the positive reactions were due to non-specific lipids resulting from tissue disintegration products in syphilis, calling forth auto-antibody formation. But their theory has not gained acceptance, because they did not present experimental evidence and because the theory did not make clear why the Wassermann reaction was not similarly applicable to diseases other than syphilis, since lipids resulting from tissue disintegration products must be present also in other diseases.

Sachs, Klopstock, and Weil (1925) thought that, in syphilis, tissue lipids in combination with spirochaetal protein formed the auto-antigen which called forth antibodies to lipids, and that these antibodies were detected *in vitro* by the use of extracts of tissue lipids. Their theory was based on experiments in which the injection of lipids plus proteins in rabbits called forth the production of antibodies to lipids, while the injection of lipids alone did not. Eagle (1937) and Eagle and Fleischman (1948) thought that the antibody responsible for serologic reactions in syphilis was a specific antibody to *T. pallidum*. Eagle assumed that this organism contains an antigenic factor immunologically similar to lipids present in the antigenic extract.

These attempts to explain positive serologic reactions in syphilis have a common weakness. They restrict the explanation to the syphilis reaction and they disregard the reactions in situations other than in syphilis. It is obvious that, for an explanation of serologic reactions with lipid antigen to be valid, syphilitic as well as non-syphilitic reactions must be clarified.

Studies by various workers (Eberson, 1921; Turner, 1939) have indicated that *T. pallidum* calls forth specific antibodies in syphilis. More recently, Nelson and Mayer (1949) have shown that the sera of persons with syphilis are able to immobilize the spirochaetes in the presence of complement. The treponemal immobilization test is practical evidence of the presence of a specific antibody against the spirochaetes in syphilis, but this test throws no light on the nature of the antibody in syphilis responsible for precipitation and complement-fixation reactions with lipid antigen.

Evidently there are two distinctive types of antibodies in syphilis. A specific antibody against the spirochaetes manifested by the immobilization test, and a special type of antibody manifested by precipitation and complement-fixation tests with lipid antigen. The latter antibody is often designated in text books as *reagin*, which by definition is an antibody or substance which behaves like an antibody. But this designation does not contribute to the knowledge of the nature of this antibody to lipids. The term *reagin* may give the incorrect impression that this antibody is fully understood.

It was seen that lipid antigen-antibody reactivity is manifested in health and is intensified in disease, after irradiation, and after the injection of foreign (non-antigenic) substances, as well as of antigenic substances. The various conditions under which lipid antigen-antibody reactivity is intensified, suggests that the biologic mechanism of this reactivity is based on tissue break-down. Such break-down, which includes normal catabolism, causes the liberation of lipids, which lipids become foreign to the body and auto-antigenic as a result of chemical changes or conjugations. Auto-antibodies are formed to these lipids which are detected *in vitro* by the universal reaction.

In view of the link existing between the universal and sero-diagnostic reactions, a concept which attempts to explain the basis of universal reactions should also apply to sero-diagnostic reactions. Accordingly, a positive sero-diagnostic reaction in syphilis is believed to be associated with tissue break-down due to this disease, and a positive sero-diagnostic reaction in the absence of syphilis is likewise believed to be associated with tissue break-down due to other causes (Kahn, 1950a, 1953; Moore and Mohr, 1952).

Only isolated individuals give positive sero-diagnostic reactions associated with tissue break-down in the absence of syphilis—those in whom lipid antigen-antibody reactivity is naturally marked and close to the sero-diagnostic zone. It is believed

that all individuals show increased lipid antigen-antibody reactivity over the normal level as a result of tissue break-down, but that in spite of the increase the reactivity is removed from the sero-diagnostic zone, so that the sero-diagnostic reactions remain negative.

Incidence of False Positives in Practice.— Because the impression might be gained from this article that false positives given by sero-diagnostic tests are common occurrences, it should be emphasized that they are, in fact, relatively rare. Evidence to this effect is shown by the Kahn results of official evaluation studies of serologic tests for syphilis carried out since 1928. These evaluations are based on the examination with sero-diagnostic tests of given numbers of blood specimens unknown to the examiner as to their origin clinically, and on the reporting of the results to an official agency. During 1928 and 1930 the official agency was the Health Organization of the League of Nations. Since 1935 the official agency has been the United States Public Health Service, aided by an advisory council. These serologic examinations are being continued and the last one was carried out in 1952. The reports are available at the V.D. Division, U.S. Publ. Hlth Service, Washington, D.C.

In the official evaluation studies since 1928, reported either by the author or by this laboratory, the Kahn gave twelve doubtful reactions, and one positive reaction on a specimen on which all other participating tests for syphilis reported a positive reaction. The total number of non-syphilitic sera employed in these evaluations was 4,138. No specimens from cases of malaria or leprosy are included in this number.

Of particular interest is the fact that the United States Public Health Service has carried out seventeen annual evaluation studies since 1937, and that in these the Kahn results reported from this laboratory did not include a single false positive or doubtful reaction. These evaluation results, covering 2,833 non-syphilitic blood specimens, are summarized in Table II.

At one time it was thought that the solution of the false positive problem might lie in the use of cardiolipin antigen. But the results of official evaluations of serologic laboratories conducted by the United States Public Health Service have shown that at least certain tests with cardiolipin antigen, in the hands of the author-serologists, are highly non-specific (Kahn and McDermott, 1953). Thus, in the 1952 evaluation, one flocculation method using cardiolipin antigen gave two false positives and nineteen false doubtful reactions in the examination

TABLE II

SPECIFICITY RECORD OF STANDARD KAHN TEST IN 25 YEARS OF OFFICIAL SEROLOGIC EVALUATIONS (1928–1952)

Based on results Reported by Author-Serologists on 4,137 Non-Syphilitic Sera

Year of Evaluation Study	Number of Sera Tested	Positive		Doubtful		Clinical Status‡
		No.	Per Cent.	No.	Per Cent.	
1928(¹)	434	0	0·0	5†	1·2	Malignancy, fever, etc.
1930(²)	304	0	0·0	2	0·7	Malignancy, fever, etc.
1935(³)	467*	0	0·0	4	0·9	Malignancy, fever, etc.
1936	99	0	0·0	1	1·0	Normal
1937–1952(⁴)	2,833	0	0·0	0	0·0	Normal, malignancy, fever, etc.

(1) Copenhagen Conf.; League of Nations' Health Organization.
(2) Montevideo Conf.; League of Nations' Health Organization.
(3) From 1935 the results are based on American Evaluation Studies.
(4) Based on seventeen different evaluations.
* In the 1935 evaluation, all participating tests gave a positive reaction with a serum from a patient with tuberculosis ; this reaction was deleted from this Table because of the likelihood of syphilis in this instance. Otherwise, the figures in the Table are as those officially listed by the Venereal Disease Division of the U.S. Public Health Service.
† The Kahn test gave a total of twelve doubtful reactions in the evaluation studies extending from 1928 to 1936. These were almost entirely with sera from persons with non-syphilitic diseases.
‡ Sera from patients with leprosy and malaria not included.

of one hundred specimens from normal, non-syphilitic donors. A complement-fixation test with cardiolipin antigen in the same one hundred specimens gave three false positives and three false doubtfuls. In the examination of the same sera by other author-serologists in that evaluation, the Kahn and some other tests employing lipoidal (non-cardiolipin) antigens gave no false positive reactions.

The specificity record of the Kahn test in official evaluation studies, although unparalleled, is not presented with the idea of suggesting that the Kahn test is not capable of giving false positives. Experience since 1923 has shown that the test is capable of giving false positives, but the data are presented as a reminder that a sero-diagnostic test for syphilis, when carried out correctly, may give results of very high specificity.

Are Universal and Sero-Diagnostic Reactions Antigen-Antibody Reactions?

The reactions between sera and lipids have been treated as antigen-antibody reactions because any other interpretation would lead to confusion. Actually there are some who believe that they are biochemical and not immunologic reactions. This question came up after the presentation of the author's papers at the Sixth International Congress for Microbiology, in September, 1953, and in answering it a number of reasons were presented

for the assumption that we are dealing here with lipid antigen-antibody reactions. That the reactions are also biochemical is not questioned, since all immunity reactions undoubtedly have a biochemical basis. A comprehensive discussion of this subject is to be published elsewhere.

Detection of False Positives

About 50 per cent. of sero-positive blood specimens reaching this laboratory from different parts of the country for verification studies are sero-negative. Both our verification and universal serologic studies indicate that, in practically all instances, the individuals from whom these blood specimens are obtained react very strongly with lipid antigen, so much so as to be close to the border of the sero-diagnostic zone. Being close to the border, even subclinical upsets may lead to temporary sero-diagnostic reactions. A positive reaction, generally weakly positive, followed in a few weeks by a negative reaction is a common occurrence in such persons. Because their serologic reactivity is quite close to the sero-diagnostic zone, these individuals are likely to give different results with different tests, depending largely on the sensitivity of the tests ; they are also likely to give fluctuating results when tested from time to time with the same test.

The limitations of the verification test as originally reported from this laboratory (Kahn, 1940b) prevented it from being the solution to the problem of the detection of false positives. In reporting the test we had two purposes in mind : to emphasize the existence of false positives and to aid physicians in detecting them.

The verification test is based on several observations that the precipitate formed with syphilitic sera is less readily dispersible than the precipitate formed with non-syphilitic sera. In certain instances of false positives, the precipitate in the standard Kahn test will disperse a few minutes after the first reading of the test. In most other instances the precipitate will disperse after the addition of a small amount, such as 0·1 ml., of 20 per cent. NaCl solution. Another characteristic of non-syphilitic reactions is the tendency for more marked precipitation to occur at a cold temperature than at 37° C. Technical details of the verification test and the method of reporting are described elsewhere (Kahn, 1950a).

The solution of the problem of aiding physicians in the detection of false positives lies in the outstanding report of Nelson and Mayer (1949) of the *Treponema pallidum* immobilization (TPI) test. In this specific test the serum from a person with syphilis immobilizes the *T. pallidum* in the presence of complement. The high complexity of the technique limits its present usefulness, but there is reason to believe that before very long these technical complexities will be sufficiently reduced to make the test more widely applicable.

The greatest value of any laboratory method lies in its employment in combination with clinical studies, and the TPI test is no exception to this rule. Thus, in a recent report on this test from the Army Medical School (Chorpenning, 1953), six positive reactions and one doubtful are recorded in 235 non-syphilitic individuals. These results do not rob the test of its outstanding value ; they emphasize merely that no laboratory method is capable of establishing a diagnosis without clinical judgment.

Summary

(1) The serology of syphilis is a branch of a biologically-universal serology with lipid antigen. All persons (and animals) examined have been found to give positive reactions with the universal serologic technique. Universal serologic reactions are constant in health, are intensified in a number of diseases studied thus far, and revert to normal on recovery. The reactions are also intensified by injections of various substances and by irradiation.

(2) Universal reactions observed thus far have shown a common serologic pattern in most instances and hence are considered to be non-specific. The serologic pattern in syphilis is distinctive and the universal reaction is considered to be lipid-specific ; essentially the same distinctive pattern has been observed also in yaws, pinta, and bejel. Another type of distinctive pattern of the universal reaction has been observed in the lepromatous form of leprosy.

(3) In syphilis the sero-diagnostic reaction is lipid-specific, since it is part of the universal reaction. Lipid-specificity is one of the factors which help to explain the high clinical value of the sero-diagnostic reaction in this disease. A second factor is the moderate activity of the disease, which is generally a forerunner of high-level antibody production, minimal activity and excessive activity being commonly forerunners of low antibody production. A third factor is the continuity of antibody production as a result of the chronic nature of the disease, and a fourth is the optimal nature of sero-diagnostic techniques in eliciting reactions in syphilis.

(4) The basis of positive sero-diagnostic reactions has been suggested by studies of the universal reaction. The fact that the universal reaction is intensified in disease, and after irradiation and on the injection of various substances, indicates that the biologic

mechanism of lipid antigen-antibody reactivity is initiated by tissue break-down, ranging from that in normal catabolism to that in disease.

(5) The forerunners of increased serologic re-activity with lipid antigen over the normal reactivity level, leading to false positive reactions, include infectious and non-infectious diseases, injections of various substances both antigenic and non-antigenic, irradiation, and apparently any condition which will result in tissue break-down over and above normal catabolism.

(6) False positive reactions are usually weak and of low quantitative titre because the techniques of sero-diagnostic tests, while favourable for the detection of syphilis, are not commonly favourable for the detection of non-syphilitic reactions. Most non-syphilitic increases in serologic reactivity, detected by the universal reaction, are not detected by sero-diagnostic reactions ; only when the increase in reactivity is unusually marked will sero-diagnostic reactions begin to show signs of positivity.

(7) The occurrence of a false positive reaction requires an incubation period of about 2 weeks after the onset of tissue break-down. Accordingly, no false positive reactions are likely to be noted during an acute disease of short duration ; if the reactions occur at all in such cases, they will be noted some 10 days to 2 weeks after recovery. False positive reactions in a chronic disease may be noted after the serologic incubation period which follows the onset of tissue break-down. In the case of injections or irradiation, the occurrence of false positive reactions should similarly be noted after the incubation period.

(8) The onset of tissue break-down may or may not correspond to the onset of clinical mani-festations, and in chronic diseases, such as leprosy, in which the clinical manifestations are of slow onset the beginning of tissue break-down and of increased serologic reactivity should theoretically occur before clinical manifestations. An instance of increased serologic reactivity in tuberculosis before clinical manifestations were seen was reported from this laboratory. Several instances of false positives before clinical manifestations were reported in lupus erythematosus by Haserick and Long (1952).

(9) A false positive reaction may persist from a few weeks to several months after the tissue break-down responsible for the reaction has come to a

standstill, since antibodies to lipids, once produced, may continue to circulate in the blood stream for varying lengths of time.

(10) Animals of different species (horses, hogs, cattle, rabbits, chickens, etc.) give relatively large numbers of positive sero-diagnostic reactions, presumably as a result of a natural tendency towards marked lipid antigen-antibody reactivity. A similar tendency is manifested in isolated instances in human beings.

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